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α-Synuclein accumulation reduces GABAergic inhibitory transmission in a model of multiple system atrophy

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ABSTRACT

Multiple system atrophy is a neurodegenerative disease caused by abnormal α-synuclein (α-syn) accumulation in oligodendrocytes and neurons. We previously demonstrated that transgenic (Tg) mice that selectively overexpressed human α-syn in oligodendrocytes exhibited neuronal α-syn accumulation. Microtubule β-III tubulin binds to endogenous neuronal α-syn to form an insoluble complex, leading to progressive neuronal degeneration. α-Syn accumulation is increased in the presynaptic terminals of Tg mice neurons and may reduce neurotransmitter release. To clarify the mechanisms underlying its involvement in neuronal dysfunction, in the present study, we investigated the effects of neuronal α-syn accumulation on synaptic function in Tg mice. Using whole-cell patch-clamp recording, we found that the frequency of miniature inhibitory postsynaptic currents was reduced in Tg mice. Furthermore, a microtubule depolymerizing agent restored normal frequencies of miniature inhibitory postsynaptic currents in Tg mice. These findings suggest that α-syn and β-III tubulin protein complex plays roles for regulation of synaptic vesicle release in GABAergic interneurons, and it causes to reduce GABAergic inhibitory transmission.

1. Introduction

Multiple system atrophy (MSA) is a neurodegenerative disease clinically characterized by autonomic nervous system failure as a symptom of Shy-Drager syndrome and parkinsonism as a symptom of striatonigral degeneration [1,2]. MSA is pathologically characterized by glial cytoplasmic inclusions (GCIs) and neuronal inclusions, both of which are composed of α-synuclein (α-syn). GCIs are the first neuropathological manifestation to be described and are oligodendrocytic inclusions [3–5]. Previous studies on GCIs have reported that filaments isolated from the central nervous system (CNS) of MSA patients were identified by using α-syn antibodies [6]. α-Syn forms the major component of the inclusions in MSA [7,8], which in the primary lesion that eventually compromises nerve cell function and viability [9]. However, the cellular mechanisms underlying MSA neurodegeneration are not fully understood, and it is unfortunate that there is no approach that provides an immediate therapeutic relief of MSA neurodegeneration.

Three transgenic (Tg) MSA mouse models in which human wild-type α-syn is overexpressed in CNS oligodendrocytes have been generated [10–12]. In our previous study, we demonstrated using a Tg mouse model that the formation of oligodendrocytic α-syn inclusions resulted in neuronal degeneration that was exemplified by motor impairments, macroscopically apparent brain atrophy, and histologically apparent decreases in the number of neurons with gliosis [11]. The accumulation of α-syn in CNS oligodendrocytes induces its neuronal accumulation and causes progressive neuronal degeneration in Tg mice. We propose that a similar disease process may underlie MSA. Accordingly, we studied novel mechanisms of neuronal α-syn accumulation in this MSA mouse model and identified a protein, microtubule β-III tubulin, that interacts with α-syn and forms an insoluble protein complex [13,14]. Furthermore, we demonstrated that the accumulation of the insoluble α-syn complex was suppressed by treatment with a microtubule depolymerizing agent [13]. These results indicate that α-syn usually binds to β-III tubulin and forms the protein complex but the abnormal increase of α-syn induces the excessive production of the protein complex, and it finally causes neuronal degeneration in the MSA mouse model [13]. The inherent function of the protein complex in neurons was unknown, but α-syn has property to aggregate itself and to bind β-III tubulin in concentration

Abbreviations: ACSF, artificial cerebrospinal fluid; CGP, (2*S*)-3-[[1*S*]-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl) phosphinic acid; CNS, central nervous system; D-APV, D-(−)-2-amino-5-phosphonopentanoic acid; DMSO, dimethyl sulfoxide; DNQX, 6,7-dinitroquinoxaline-2,3(1H,4H)-dione; $GABA_A$, γ-aminobutyric acid type A; GCI, glial cytoplasmic inclusion; mEPSC, miniature excitatory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current; mPFC, medial prefrontal cortex; MSA, multiple system atrophy; α-syn, α-synuclein; Tg, transgenic; TTX, tetrodotoxin.

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dependent manner [14]. Thus, it suggests that mechanism to properly regulate the formation of the protein complex may exist. Neuronal activity controls the synaptic accumulation of α-syn [15]. Accumulation of α-syn in Tg mice was unaltered by treatment with the γ-aminobutyric acid type A $(GABA)_A$ picrotoxin, whereas soluble α-syn decreased following this treatment in wild-type mice [13]. Because α-syn accumulation was increased in the presynaptic terminals of Tg mice [11], this accumulation may reduce neurotransmitter release. In the present study, we used electrophysiological approaches to assess whether normal synaptic function was disturbed by α-syn accumulation and to further clarify the mechanisms responsible for neuronal dysfunction in MSA.

2. Materials and methods

2.1. Animals and nocodazole treatment

Tg mice that expressed human α-syn in CNS oligodendrocytes, driven by the murine CNP promoter, were generated as described previously [11,16]. Electrophysiological experiments were carried out in Tg and wild-type mice. For the experiment designed to record miniature inhibitory postsynaptic currents (mIPSCs), the mice ranged in age from 4 to 12 weeks. For the experiment designed to record miniature excitatory postsynaptic currents (mEPSCs), the mice ranged in age from 4 to 12 weeks. In some experiments, the control Tg mice were administered either 1 μM of nocodazole (Sigma–Aldrich, MO, USA) or an equal amount of dimethyl sulfoxide (DMSO, Kanto Chemical, Tokyo, Japan). Both nocodazole and DMSO were administered orally, via drinking water, from postnatal day 1 to the day of the electrophysiological experiments, and wild-type mice were also administered the same amount of DMSO administered to the control Tg mice. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and they were approved by the Institutional Animal Care and Use Committee at the National Center for Geriatrics and Gerontology.

2.2. Electrophysiological/pharmacological experiments

Mice were deeply anesthetized by fluothane inhalation and then sacrificed by decapitation. Their brains were removed, and 300 μm coronal slices containing the medial prefrontal cortex (mPFC) were prepared immediately. These slices were then placed in an interface holding chamber filled with a humid gas mixture of 95% O_2 and 5% CO_2 to equilibrate for at least 1 h before recording. For recording, the slices were transferred to a submerged-type recording chamber and perfused at a rate of 2 ml/min with artificial cerebrospinal fluid (ACSF) saturated with 95% O_2 and 5% CO_2 and containing (in mM) 120 NaCl, 3 KCl, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, and 15 glucose.

To record mIPSCs and mEPSCs, whole-cell patch-clamp recordings were performed on layer II/III neurons of the mPFC using an amplifier (Multiclamp 700B; Axon Instruments, CA, USA) at 28 ± 1 °C. Layer II/III neurons were oval or pyramidal with prominent dendritic shafts extending toward the pial surface, and their relatively large soma size and appearance suggested that they were probably the principal pyramidal neurons. For the mIPSC recordings, slices were perfused with ACSF containing 1 μM tetrodotoxin (TTX, Sigma–Aldrich), 10 μM 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (DNQX, Sigma–Aldrich), and 50 μM D-(−)-2-amino-5-phosphonopentanoic acid (D-APV, Sigma–Aldrich) to block voltage-dependent Na^+ channels and glutamatergic excitatory synaptic currents. Similarly, slices were perfused with ACSF containing TTX, 2 μM bicuculline (Sigma–Aldrich), and 100 nM (2*S*)-3-[[1*S*]-1-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl](phenylmethyl) phosphinic acid (CGP) 55845 (Tocris Cookson, UK) to block voltage-dependent Na^+ channels and GABAergic inhibitory synaptic currents. Glass recording pipettes (4–6 MΩ tip resistance) for recording mIPSCs were filled with an internal solution containing (in mM) 130 CsCl, 0.5 $CaCl_2$, 2 $MgCl_2$, 1 EGTA, 10 HEPES, 2 Mg-ATP, and 2 QX-314 (adjusted to pH 7.2 with CsOH). Glass recording pipettes (4–6 MΩ tip resistance) for recording mEPSCs were filled with a different internal solution containing (in mM) 135 Cs-methanesulfonate, 8 NaCl, 0.3 EGTA, 10 HEPES, 4 Mg-ATP, 0.3 Na_3-GTP, 5 QX314, and 0.1 spermine (adjusted to pH 7.2 with CsOH). The holding membrane potential was set to −70 mV or −80 mV for recording mIPSCs and mEPSCs, respectively. Under these experimental conditions, both chloride currents due to $GABA_A$ receptors and cation currents due to ionotropic glutamate channels were inward currents. Series resistance was in the range of 20–30 MΩ, and the data were discarded if the resistance changed to >30% during a recording. Signals were filtered at 2 kHz and sampled at 5 kHz. mIPSCs and mEPSCs were visually inspected offline, and peak amplitudes were analyzed using Mini Analysis software (Synaptosoft, GA, USA).

2.3. Statistical analysis

Statistical significance between the wild-type and Tg groups was calculated using the Mann–Whitney *U*-test, unless otherwise noted. The differences between multiple groups were evaluated by the Steel–Dwass test. Data for the Mann–Whitney *U*-test and Steel–Dwass test were expressed as median values (interquartile ranges). All other data were presented as the mean ± standard error of the mean. In all cases, $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Reduced inhibitory synaptic transmission in Tg mice

A previous study revealed that GCI-like inclusions of human α-syn in oligodendrocytes led to an age-dependent accumulation of endogenous mouse α-syn in neuronal axons and nerve terminals of the CNS of Tg mice [11]. Because α-syn is a negative regulator of neurotransmitter release [17], we tested whether the upregulation of endogenous α-syn reduces neurotransmitter release in Tg mice. To examine excitatory transmission in Tg mice, miniature excitatory postsynaptic currents (mEPSCs) were recorded using whole-cell patch-clamp recordings in the presence of TTX, bicuculline, and CGP 55845. In 4-week-old mice, there were no differences in the averaged frequency of mEPSCs [wild-type, 4.7 (3.3–6.0) Hz, $n = 8$ neurons/5 mice; Tg, 4.6 (4.3–6.5) Hz, $n = 7$ neurons/3 mice; $p = 0.6$] and in the averaged amplitude of mEPSCs [wild-type, 12.1 (11.7–13.2) pA, $n = 8$ neurons/5 mice; Tg, 12.5 (11.9–13.4) pA, $n = 7$ neurons/3 mice; $p = 0.7$]. The frequencies of mEPSCs in 8-week-old Tg mice were similar to those in wild-type mice (Fig. 1A). An accumulated histogram from the pooled data showed no difference in the distribution of either the inter-event intervals or the amplitudes (Figs. 1B and C). The averaged frequencies of mEPSCs in Tg mice were similar to those in wild-type mice [wild-type, 4.0 (2.9–6.1) Hz, $n = 16$ neurons/6 mice; Tg, 4.6 (2.7–6.7) Hz, $n = 17$ neurons/5 mice; $p = 0.5$; Fig. 1D]. The averaged amplitudes in Tg mice were also similar to those in wild-type mice [wild-type, 11.1 (8.9–11.8) pA, $n = 16$ neurons/6 mice; Tg, 10.3 (8.9–12.2) pA, $n = 17$ neurons/5 mice; $p = 1.0$; Fig. 1E]. In 12-week-old mice, there were no differences in the averaged frequency of mEPSCs [wild-type, 4.7 (3.3–5.5) Hz, $n = 10$ neurons/4

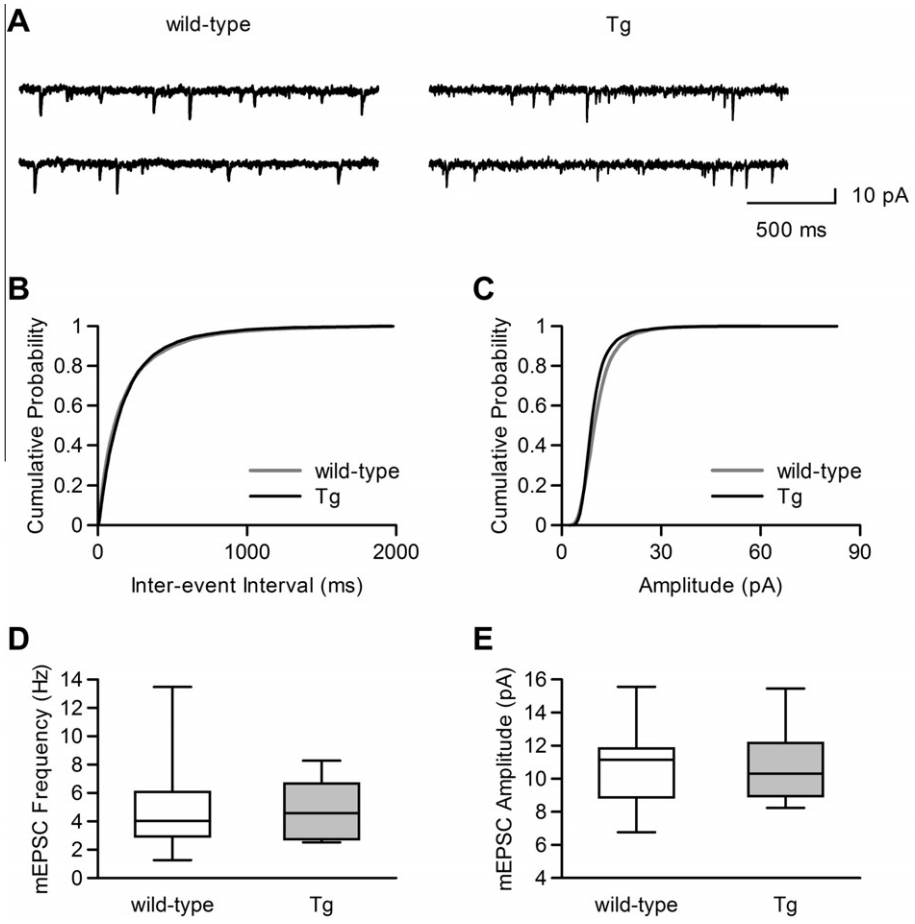

Fig. 1. Miniature excitatory postsynaptic currents (mEPSCs). (A), Representative mEPSC traces from wild-type and Tg mice. Cumulative histograms of inter-event intervals (B) and amplitudes (C) of mEPSCs recorded from pooled data from all experiments and averaged frequency (D) and amplitude (E) data of mEPSCs from each experiment are shown. There were no differences in the averaged frequency (D) and in the averaged amplitude (E). The statistical analyses are presented as box-and-whiskers plots. The horizontal lines in the box denote the 25th, 50th, and 75th percentile values. The error bars denote the lowest and the highest value.

mice; Tg, 3.9 (3.7–5.7) Hz, $n = 9$ neurons/3 mice; $p = 0.8$] and in the averaged amplitude of mEPSCs [wild-type, 10.4 (9.1–12.2) pA, $n = 10$ neurons/4 mice; Tg, 11.8 (9.6–13.4) pA, $n = 9$ neurons/3 mice; $p = 0.2$].

To examine GABAergic inhibitory transmission in Tg mice, miniature inhibitory postsynaptic currents (mIPSCs) were recorded in the presence of TTX, DNQX, and D-APV. In 4-week-old mice, there were no differences in the averaged frequency of mIPSCs [wild-type, 5.4 (4.1–7.1) Hz, $n = 11$ neurons/5 mice; Tg, 4.5 (3.5–6.7) Hz, $n = 13$ neurons/5 mice; $p = 0.4$] and in the averaged amplitude of mIPSCs [wild-type, 32.1 (25.3–41.1) pA, $n = 11$ neurons/5 mice; Tg, 24.4 (13.8–24.8) pA, $n = 13$ neurons/5 mice; $p = 0.2$]. In contrast, we confirmed that 8-week-old Tg mice exhibited decreased mIPSC frequencies (Fig. 2A). A cumulative histogram from the pooled data shows a difference in the distribution of the inter-event intervals (Fig. 2B), with no changes in the current amplitudes (Fig. 2C). The frequencies of mIPSCs, averaged from each experiment, were reduced in Tg mice [wild-type, 4.8 (4.0–8.0) Hz, $n = 14$ neurons/8 mice; Tg, 2.9 (2.5–4.3) Hz, $n = 15$ neurons/8 mice; $p = 0.006$; Fig. 2D], with no changes in the mIPSC amplitudes [wild-type, 19.8 (17.4–23.5) pA, $n = 14$ neurons/8 mice; Tg, 18.5 (14.5–24.7) pA, $n = 15$ neurons/8 mice; $p = 0.5$; Fig. 2E]. In 12-week-old mice, there was significant difference in the averaged frequency of mIPSCs [wild-type, 3.6 (3.4–5.3) Hz, $n = 9$ neurons/4 mice; Tg, 2.3 (1.0–3.5) Hz, $n = 11$ neurons/4 mice; $p = 0.03$] and no difference in the averaged amplitude of mIPSCs [wild-type, 13.5 (12.3–14.5) pA, $n = 9$ neurons/4 mice; Tg, 13.4 (9.4–28.9) pA, $n = 11$ neurons/4 mice; $p = 0.8$]. These results suggest that inhibitory synaptic transmission was reduced in Tg mice.

3.2. Microtubule depolymerization rescues GABAergic dysfunction

α-Syn accumulation increased in the neuronal presynaptic terminals of Tg mice, leading to neuronal degeneration [11,13]. To investigate the effect of nocodazole, a microtubule depolymerizing agent, on the synaptic function in Tg mice, mIPSCs were recorded in the presence of TTX, DNQX, and D-APV. Nocodazole-treated Tg (nocodazole Tg) mice exhibited more frequent mIPSCs than DMSO-treated Tg (DMSO Tg) mice. In contrast, there were no differences in mIPSC frequencies between the nocodazole Tg mice and DMSO-treated wild-type (DMSO wild-type) mice (Fig. 3A). A cumulative histogram from the pooled data showed a difference in the distribution of the inter-event intervals (Fig. 3B), but no differences in the current amplitudes. Nocodazole Tg mice exhibited higher mIPSC frequencies, averaged from each experiment, than DMSO Tg mice. We found no differences in the mIPSC frequencies between the nocodazole Tg and DMSO wild-type mice [DMSO wild-type, 4.1 (2.9–5.5) Hz, $n = 17$ neurons/5 mice; DMSO Tg 3.0 (1.9–3.8) Hz, $n = 19$ neurons/4 mice; nocodazole Tg, 4.1 (3.5–5.0) Hz, $n = 17$ neurons/4 mice; DMSO wild-type versus DMSO Tg, $p = 0.04$; DMSO Tg versus nocodazole Tg, $p = 0.03$; DMSO wild-type versus nocodazole Tg, $p = 1.0$;

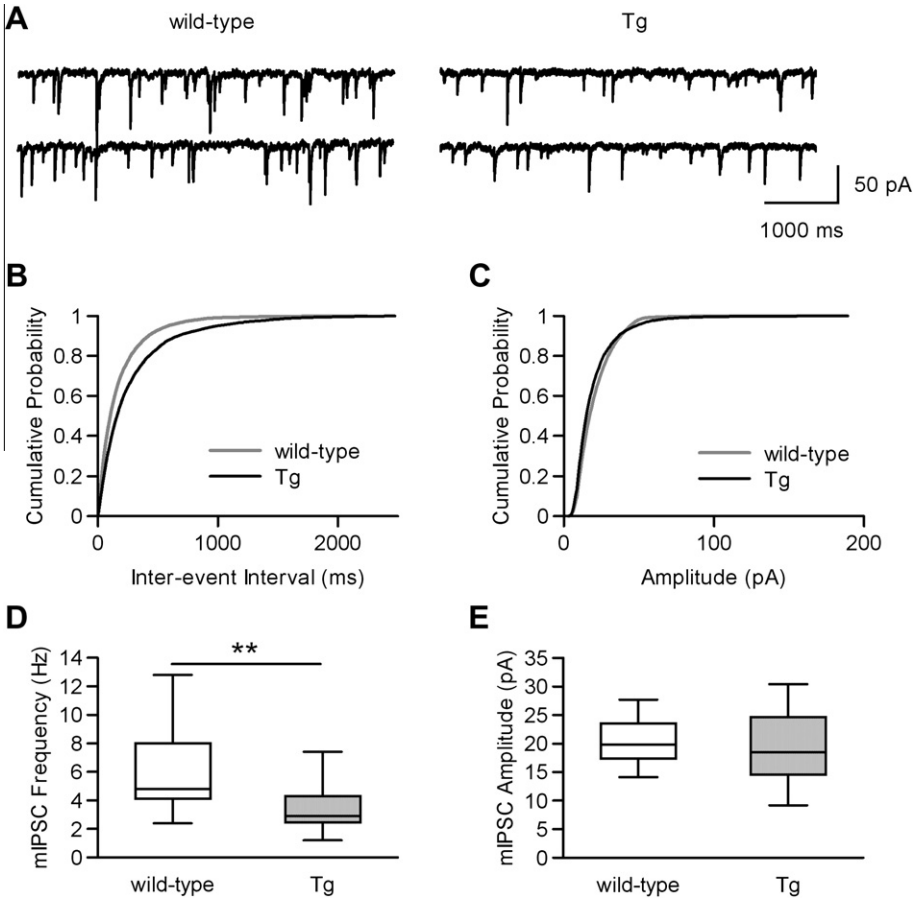

Fig. 2. Reduced inhibitory synaptic transmission in transgenic (Tg) mice. (A), Representative miniature inhibitory postsynaptic current (mIPSC) traces from wild-type and Tg mice are shown. Cumulative histograms of inter-event intervals (B) and amplitudes (C) of mIPSCs recorded from pooled data from all experiments and averaged frequency (D) and amplitude (E) data of mIPSCs from each experiment are shown. There was significant difference in the averaged frequency (D), but no difference in the averaged amplitude (E). $^{**}p < 0.01$ by the Mann–Whitney *U*-test. Box-and-whisker graphs are presented, as in Fig. 1.

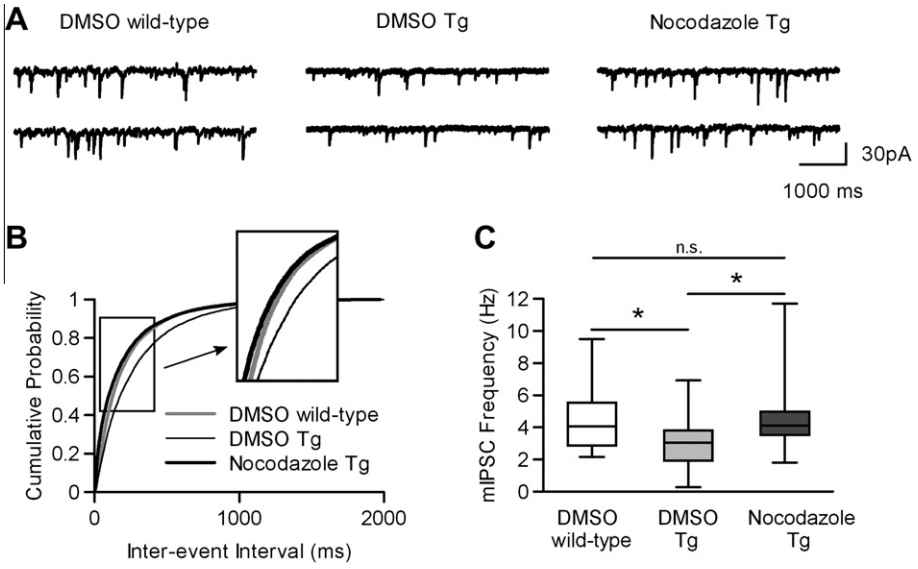

Fig. 3. Effects of a microtubule depolymerizing agent on mIPSCs. (A), Representative mIPSC traces from DMSO-treated wild-type (DMSO wild-type), DMSO-treated Tg (DMSO Tg), and nocodazole-treated Tg (nocodazole Tg) mice are shown. Cumulative histograms of inter-event intervals of mIPSCs recorded from pooled data from all experiments (B) and a comparison of the frequencies of mIPSCs from several experiments are shown (C). The frequency was significantly smaller in DMSO Tg mice compared to DMSO wild-type mice, but it was not different between nocodazole Tg mice and DMSO wild-type mice (DMSO wild-type versus DMSO Tg, $p = 0.04$; DMSO Tg versus nocodazole Tg, $p = 0.03$; DMSO wild-type versus nocodazole Tg, $p = 1.0$) (C). $^{*}p < 0.05$ by the Steel–Dwass test. Box-and-whisker graphs are presented, as in Fig. 1.

Fig. 3C]. The data demonstrated that GABAergic dysfunction in Tg mice was attenuated by depolymerization of microtubules, indicating that the binding of α-syn to β-III tubulin was a key process in the development of MSA-relevant neuronal dysfunc- tion.

4. Discussion

In the present study, we demonstrated that neuronal α-syn accumulation induced age-related loss of synaptic function in a mouse model. Because the frequency of GABAergic mIPSCs was reduced in Tg mice with no alteration in the frequency of glutamatergic mEPSCs, it appears that the inhibitory transmission was reduced in Tg mice, but excitatory transmission remained unchanged. Our data suggested that GABAergic inhibition was reduced by α-syn accumulation in Tg mice. Furthermore, a microtubule depolymerizing agent attenuated the reductions in inhibitory transmission, and reduced the accumulation of α-syn in Tg mice.

The frequency of mIPSCs in layer II/III of mPFC neurons was reduced in Tg mice, suggesting that the presynaptic GABAergic interneuron activity is attenuated. In a previous study, we found that Tg mice selectively overexpressing human α-syn in oligodendrocytes exhibited endogenous α-syn accumulation in neurons, and α-syn accumulation was observed in the neuronal presynaptic terminals of Tg mice [11]. α-Syn is a small (140 amino acid), soluble neuronal cytoplasmic protein that localizes predominantly to the presynaptic terminals in the CNS, where it is associated with neurotransmitter release [17–21]. Although the normal functions of α-syn remain unclear, α-syn overexpressing mice exhibit reduced neurotransmitter release [22,23]. In contrast, α-syn knockout mice show little effects on neurotransmitter release [24,25]. These data indicate that one of functions of α-syn may be negative regulation of synaptic vesicles, which leads to decreased neurotransmitter release. Recently, overexpression of α-syn in Tg mice diminished the expression of several exocytic and endocytic endogenous presynaptic proteins, leading to neurotransmitter release deficits [26]. The studies support the hypothesis that GABAergic interneuron activity is attenuated by presynaptic dysfunction due to endogenous neuronal α-syn accumulation in Tg mice. In the present study, we found that neuronal α-syn accumulation caused a reduction in the presynaptic GABA release, even before the actual degeneration of GABAergic interneurons.

GABAergic dysfunction in Tg mice was suppressed by depolymerization of microtubules. α-Syn has been reported to regulate monoamine transporters by interacting with microtubules, and nocodazole enhanced cell surface expression of monoamine transporters [27]. Endogenous mouse α-syn in neurons, which is bound to β-III tubulin in microtubules, was reduced by nocodazole treatment [13,14]. Other results show that intranasal administration of microtubule-interacting peptide in overexpressing α-syn Tg mice reduces α-syn inclusions [28]. These studies indicate that the binding of α-syn to β-III tubulin is a key process in the development of neuronal degeneration in the MSA mouse models. In the present study, we demonstrated that the reduction in the presynaptic GABAergic interneuron activity was due to neuronal accumulation of α-syn bound to β-III tubulin. Our findings suggest a possibility that the neuronal accumulation of α-syn bound to β-III tubulin reduces the presynaptic GABAergic interneuron activity, leading to decreased neurotransmitter release in a mouse model of MSA. Furthermore, synaptic dysfunction is prevented by chronic treatment with a microtubule depolymerizing agent before α-syn accumulation. This study enhances our understanding of the mechanisms that underlie neurodegeneration observed in MSA patients and provides a novel therapeutic strategy for this disorder.

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